

Fig. 1. TCR $\alpha\beta$ Transgene Expression in 8.3-NOD Mice. CD4/CD8/V β 8.1/8.2 profiles of thymocytes (A) and lymph node cells (B) from non-transgenic-, 8.3 TCR β -transgenic-, and 8.3NOD mice. DP, double-positive; DN, double-negative.

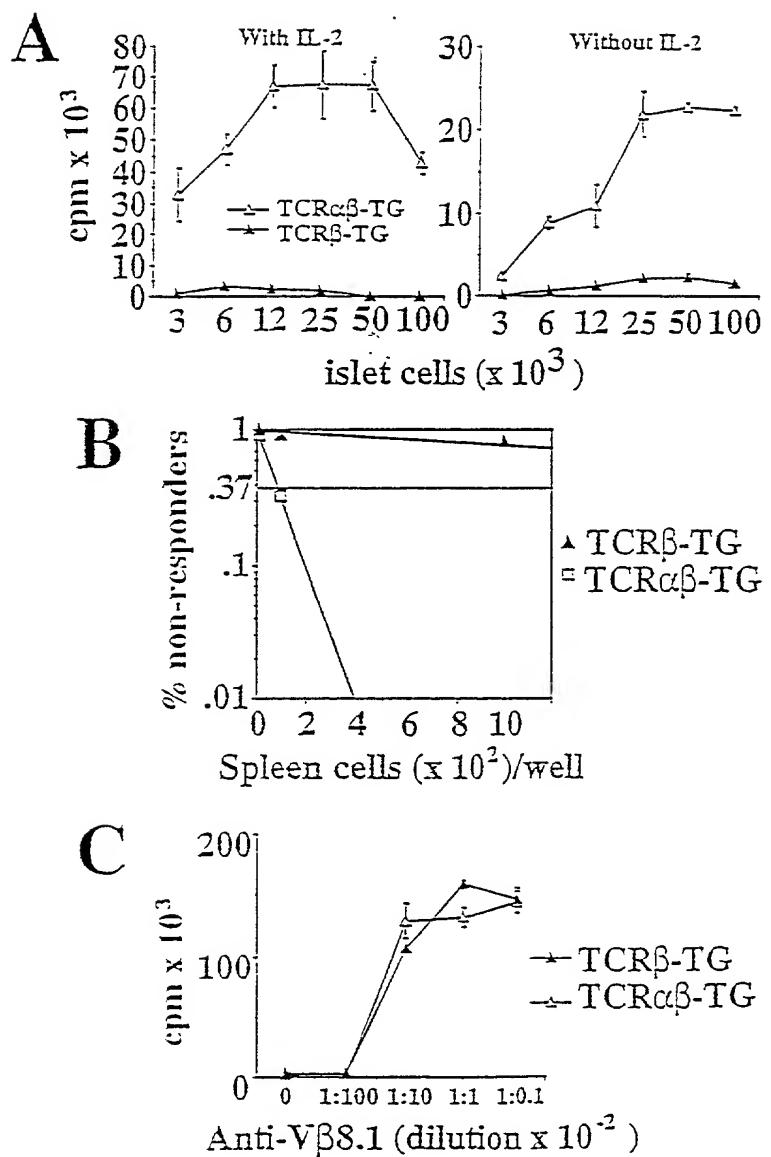


Fig. 2. Beta Cell-Specific CD8+ T-Cells in 8.3-NOD Mice. (A) Proliferation of splenic CD8+ T-cells to islet cells. (B) Peripheral frequency of beta cell-reactive CD8+ T-cells. (C) General proliferative activity of splenic CD8+ T-cells.

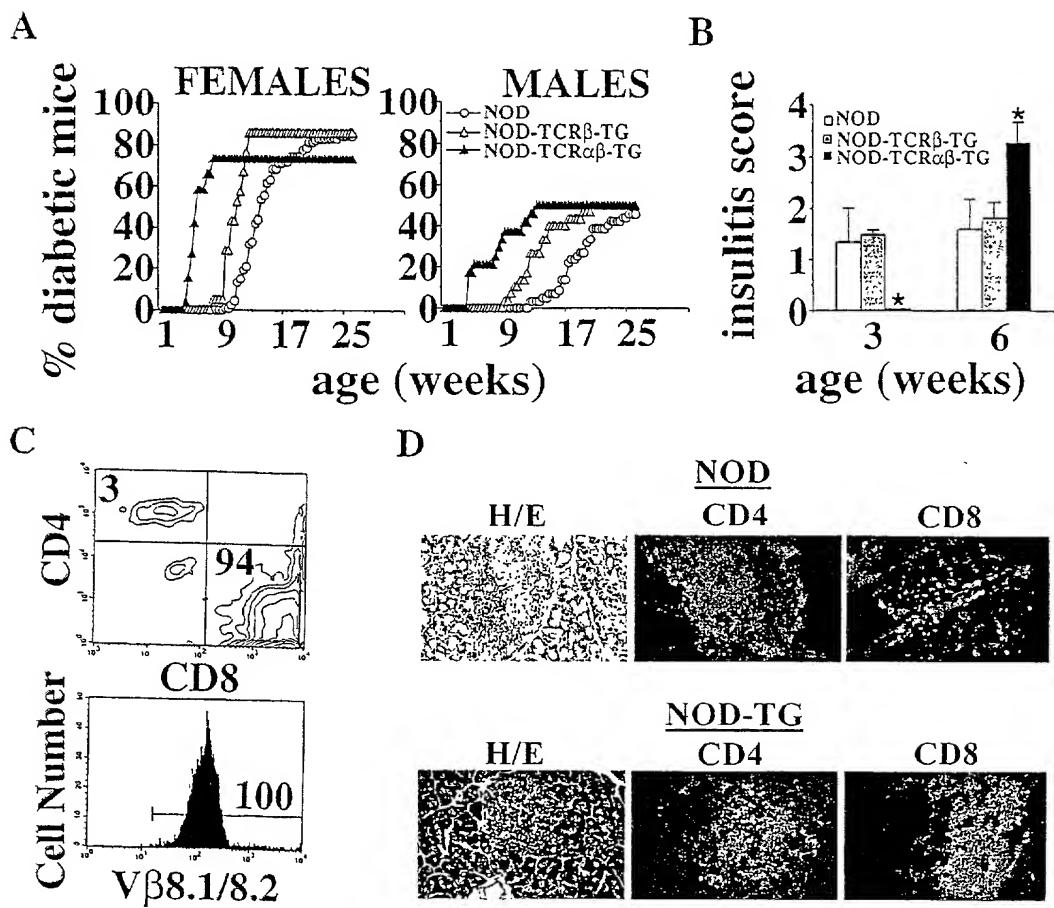


Fig. 3. 8.3-TCR $\alpha\beta$ -Transgene Expression and Diabetogenesis. (A) Incidence of IDDM. *, p<0.0001 (χ^2). (C) Flow cytometry profile of islet-derived T-cells from diabetic 8.3-NOD mice. (D) Phenotype of islet-infiltrating T-cells in 8.3-NOD vs. non-transgenic NOD mice.

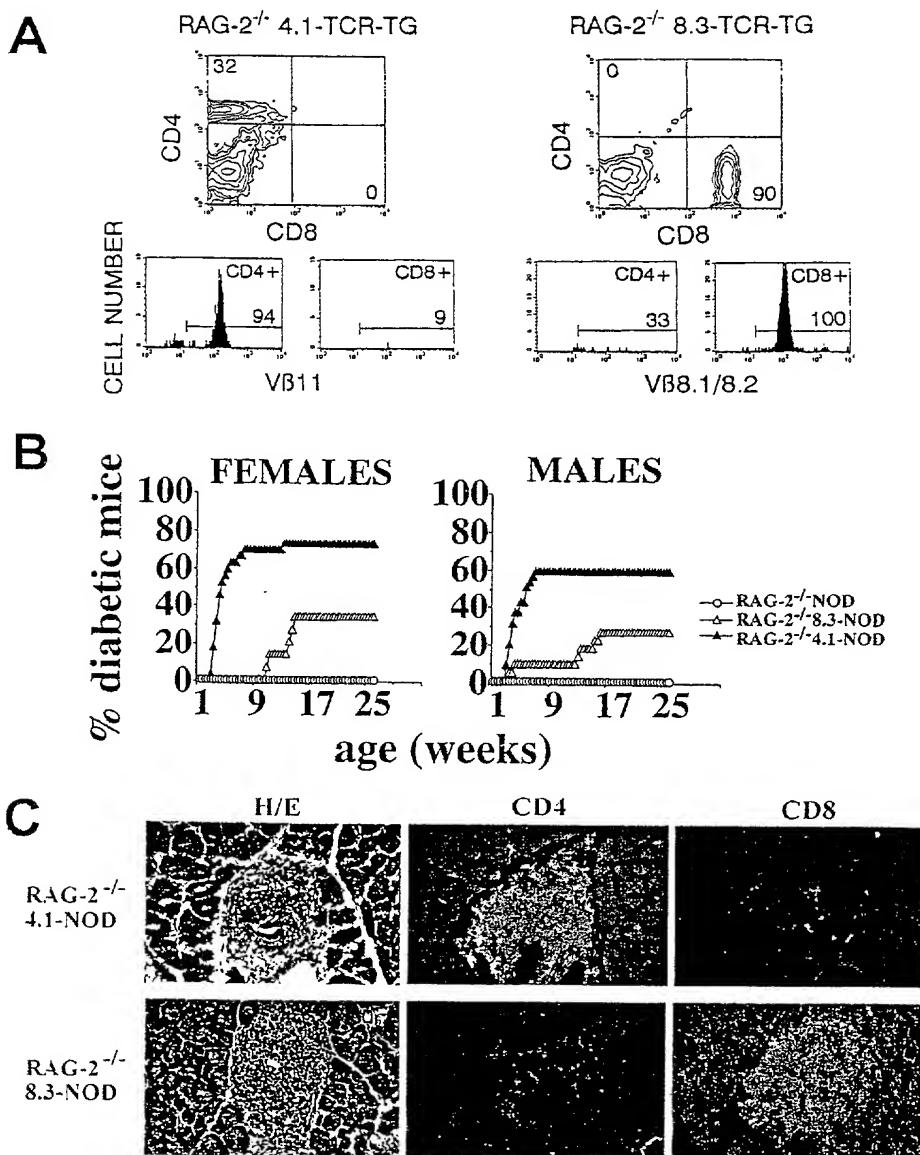


Fig. 4. Diabetogenesis in Monoclonal T-Cell NOD Mice. (A) FACS profiles of lymph node cells. (B) IDDM incidence. (C) Phenotype of insulitis T-cells. Most of the few CD8⁺ T-cells in RAG-2^{-/-} 4.1-NOD mice, and the few CD4⁺ T-cells in RAG-2^{-/-} 8.3-NOD mice are due to background staining, as they are also seen in anti-rat IgG-FITC-stained tissue.

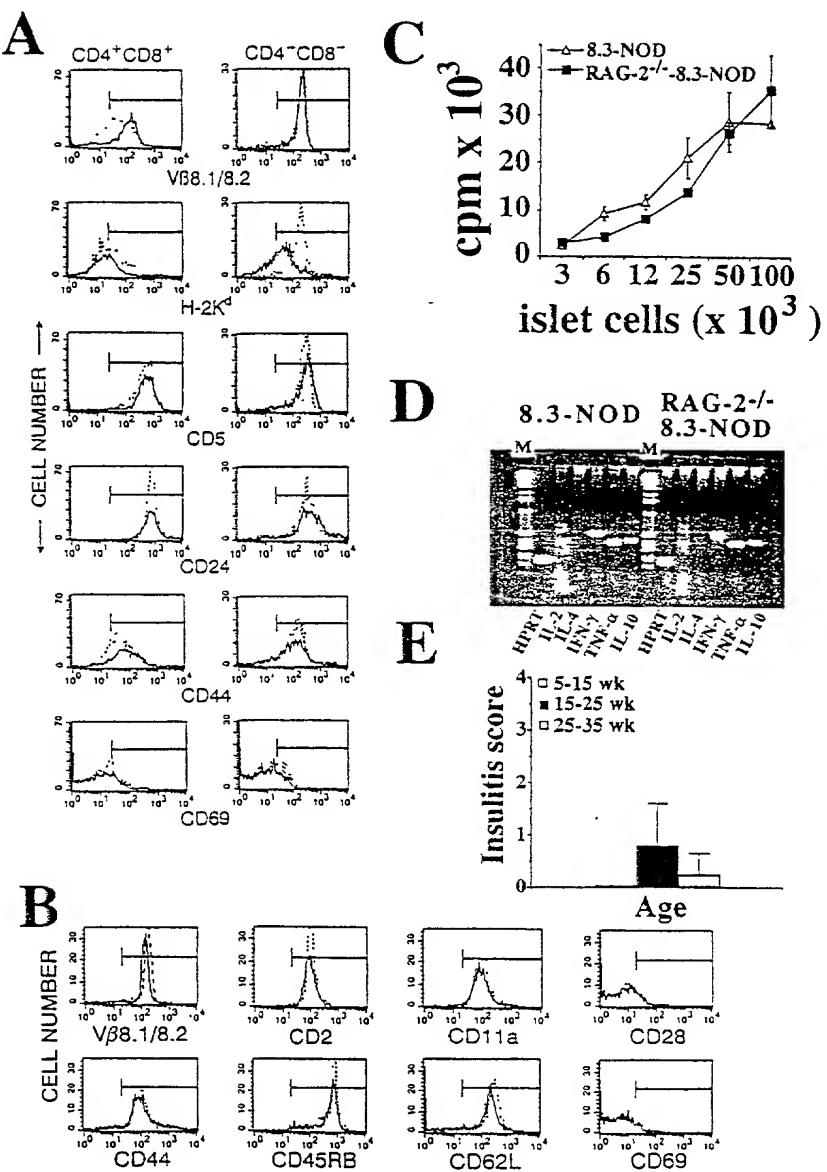


Fig. 5. Phenotypic and Functional Analysis of CD8+ T-cells from RAG-2- 8.3-NOD Mice. (A) Maturation markers on thymocyte subsets from 8.3-NOD (dotted line) and RAG-2-/- 8.3-NOD mice (solid line). (B) Activation/memory markers on splenic CD8+ T-cells from 8.3-NOD mice (dotted line) and RAG-2-/- 8.3-NOD mice (solid line). (C) Proliferative activity of splenic CD8+ T-cells in response to islet cells. (D) Cytokine RT-PCR analysis of islet-derived CD8+ T-cells. (E) Kinetics of insulitis in RAG-2-/- 8.3-NOD mice.

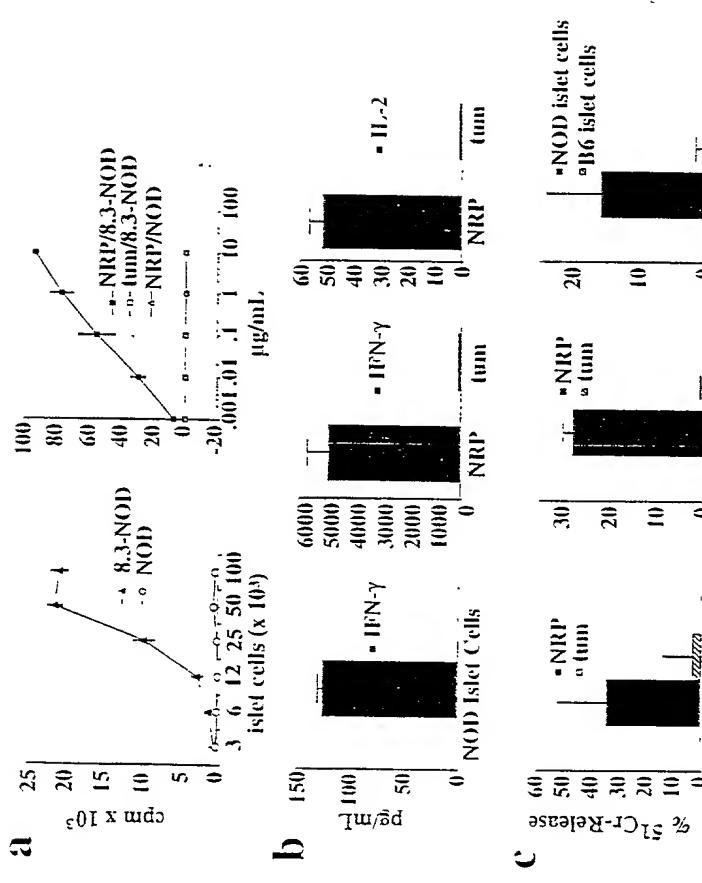


Fig. 6. Immunological Properties of NRP. **A.** Proliferation of CTLp from 8.3-NOD and NOD/Lt mice in response to NOD islet-cells (left) or NOD splenocytes pulsed with NRP or tum (right) ($p<0.05$ for islet cell-line vs. NRP) or 8.3-CTLp, and NRP-induced proliferation of 8.3-CTLp vs. NOD/Lt T-cells. **B.** Cytokine secretion by 8.3-CTLp in response to NOD islet cells or splenocytes pulsed with 1 μ g/ml of NRP or tum (middle and right panels) ($p<0.009$ for IFN- γ vs. tum). **C.** Differentiation of 8.3-CTLp from RAG-2 $^{-/-}$ 8.3-NOD mice into CTL. The panel shows ^{51}Cr -release assays using NRP- or tum-pulsed RMA-SKd cells (left and middle panels) or NOD or B6 islet cells as targets, at a 1:10 T:E ratio (right) ($p<0.05$ for NRP- vs. tum-reactivity or NOD- vs. B6 islet cell-reactivity).

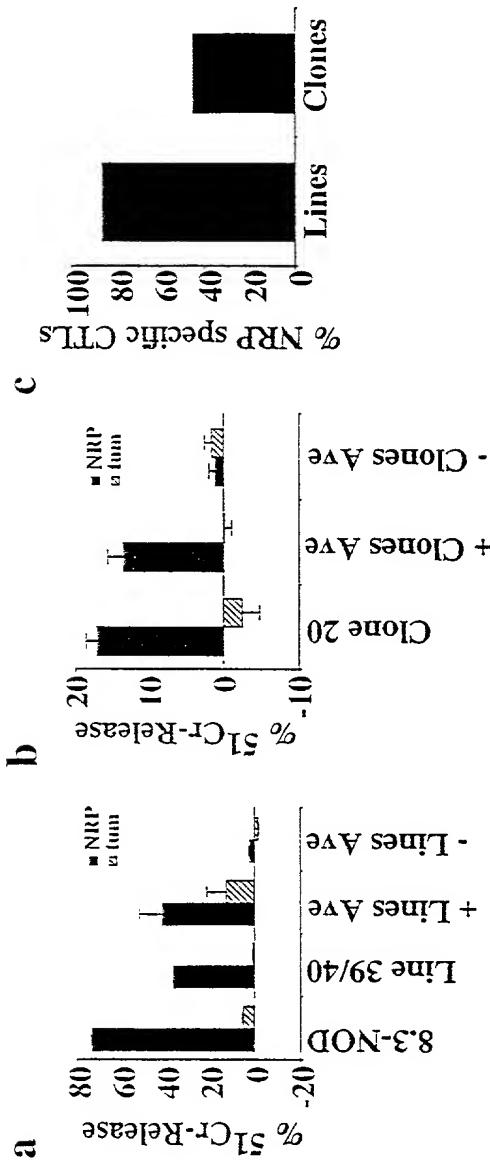


Fig. 7. NRP-reactivity of islet-associated CD8+ T-cells from NOD/Lt mice. A, NRP- and tum-reactivity of islet- and spleen-derived CD8+ T-cell lines from 8.3-NOD and/or NOD/Lt mice. CD3-act. are control CD8+ CTLs generated by stimulation of NOD splenic CD8+ T-cells with plate-bound anti-CD28 and anti-CD3 mabs. The Fig. shows results of cytotoxicity assays obtained with 1 islet line, 2 splenic lines, and the average values obtained with 7 NRP-reactive and 1 non-reactive islet lines (at 1:10 T:E ratio) ($p<0.03$). The tum reactivity of the +Lines was due to one line which displayed some cytotoxicity against tum-pulsed targets. B, NRP-reactivity of islet-derived CD8+ T-cell clones from NOD/Lt mice. Assays were done at a 1:1 T:E ratio. A clone was defined as positive if it triggered 51Cr-release values from NRP-pulsed targets at least 2 SD above those triggered by tum-pulsed targets. The Fig. shows results of assays obtained with 1 clone, 2 SD above those triggered by tum-pulsed target. The Fig. shows results of assays obtained with 1 clone, and average values corresponding to 14 NRP-reactive clones ($p<0.004$ for NRP- vs. tum-reactivity) and 17 non-NRP-reactive clones, C, % of NRP-reactive CTL lines and clones from NOD/Lt mice and clones from NOD/Lt mice (p<0.0001 for % of NRP- vs. tum-reactive clones).

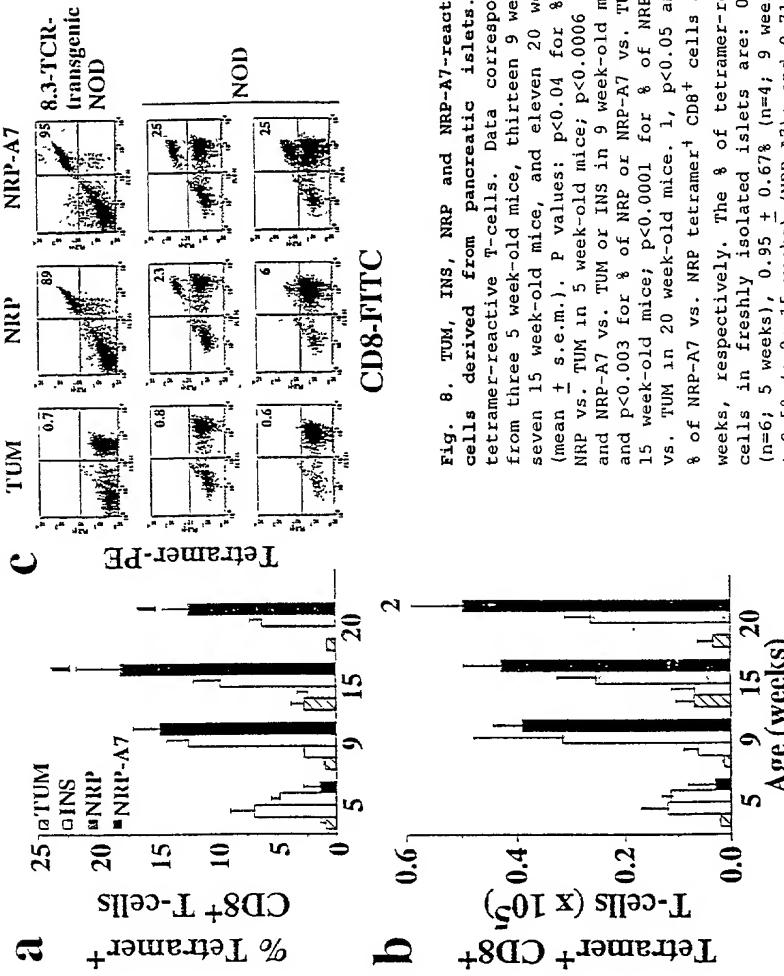


Fig. 8. TUM, INS, NRP and NRP-A7-reactive CD8⁺ T-cells derived from pancreatic islets. **A**, % of tetramer-reactive T-cells. Data correspond to cells from three 5 week-old mice, thirteen 9 week-old mice, seven 15 week-old mice, and eleven 20 week-old mice (mean \pm s.e.m.). P values: p<0.04 for % of INS and NRP vs. TUM in 5 week-old mice; p<0.0006 for % of NRP and NRP-A7 vs. TUM or INS in 9 week-old mice; p<0.013 and p<0.003 for % of NRP or NRP-A7 vs. TUM or INS in 15 week-old mice; p<0.0001 for % of NRP and NRP-A7 vs. TUM in 20 week-old mice. 1, p<0.05 and 0.008 for % of NRP-A7 vs. NRP tetramer⁺ CD8⁺ cells at 15 and 20 weeks, respectively. The % of tetramer-reactive CD8⁺ cells in freshly isolated islets are: 0.13 \pm 0.38% (n=6; 5 weeks), 0.95 \pm 0.67% (n=4; 9 weeks), and 1.6 \pm 0.5% (n=2; 15 weeks) (NRP-A7); and 0.71 \pm 0.4 (n=4; 5 weeks) and 0.31 \pm 0.4 (n=4; 9 weeks) (INS). **B**, Absolute number of tetramer-reactive T-cells from the same samples as in a (mean \pm s.e.m.). P values: p<0.04 for NRP-A7 at 15 and 20 weeks vs. 9 and 5 weeks; p<0.001 for NRP and NRP-A7 vs. TUM at 9 weeks; p<0.035 for NRP or NRP-A7 vs. INS at 9 weeks; p<0.037 for NRP or NRP-A7 vs. TUM or INS at 15 weeks; p<0.0001 for NRP or NRP-A7 vs. TUM at 20 weeks. 2, p<0.05 NRP-A7 vs. NRP at 20 weeks. **C**, Representative tetramer staining patterns. Over 90% of the CD8⁺ negative cells shown in the plots are CD4⁺.

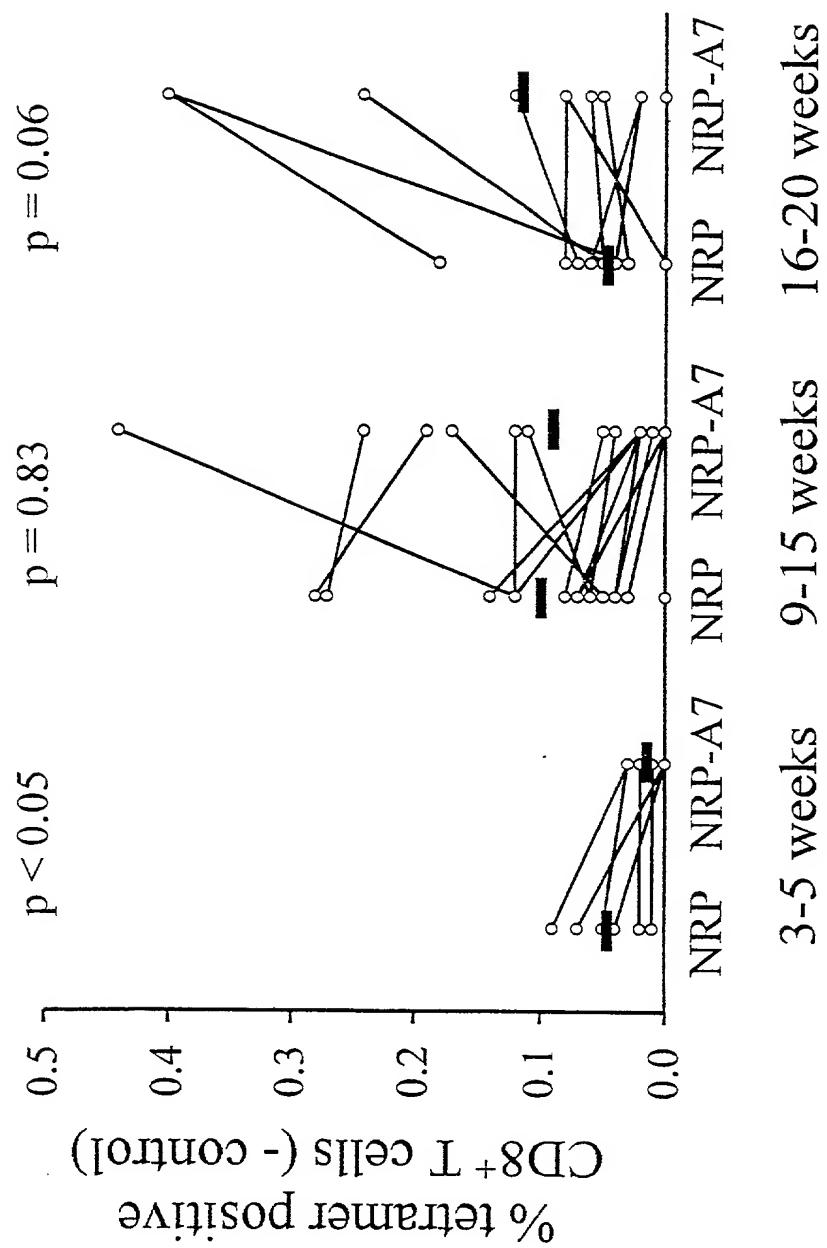


Fig. 9. Percentage of tetramer-positive CD8⁺ T-cells in pancreatic lymph nodes of NOD mice (data from J. Trudeau and R. Tan).

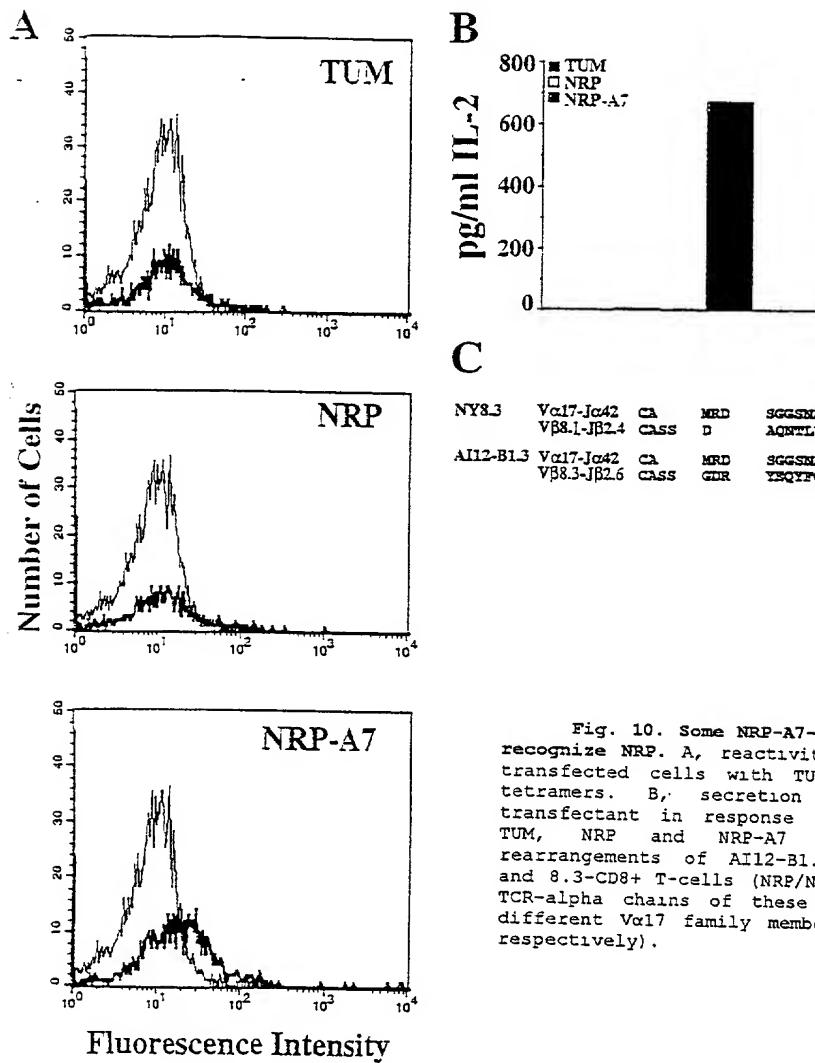


Fig. 10. Some NRP-A7-reactive TCRs do not recognize NRP. A, reactivity of AI12-B1.3 TCR-transfected cells with TUM, NRP and NRP-A7-tetramers. B, secretion of IL-2 by the transfectant in response to stimulation with TUM, NRP and NRP-A7 peptides. C, TCR rearrangements of AI12-B1.3 (NRP-A7-reactive) and 8.3-CD8+ T-cells (NRP/NRP-A7-reactive). The TCR-alpha chains of these two clonotypes use different V α 17 family members (17.5 and 17.4, respectively).

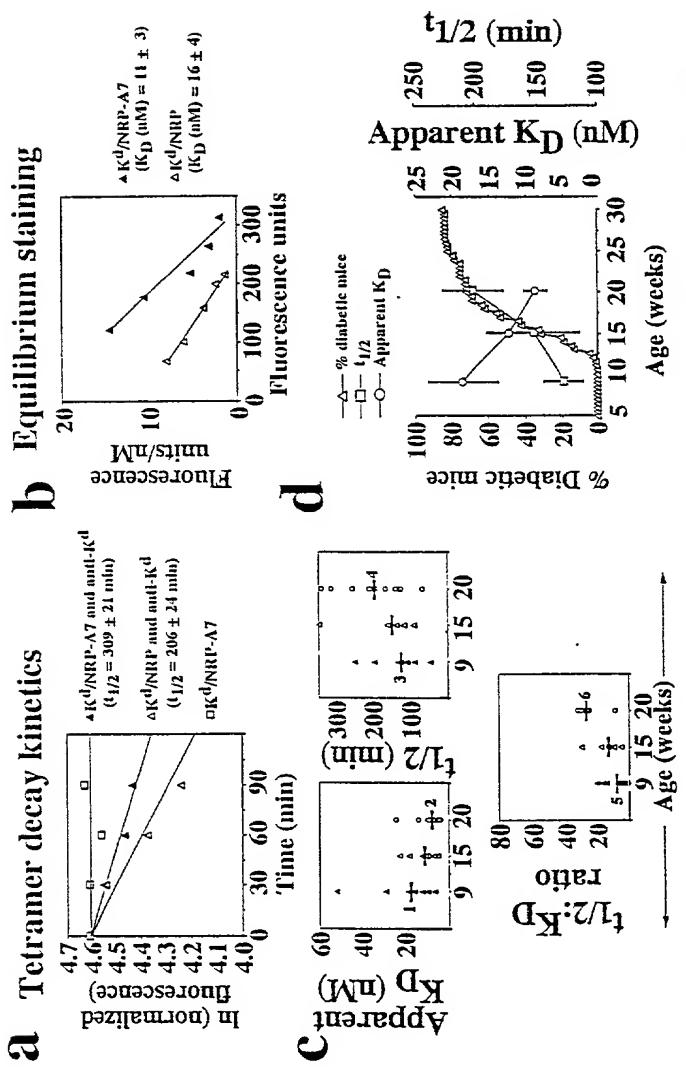


Fig. 11. Association and dissociation kinetics of NRP and NRP-A7 tetramers. **A**, Tetramer decay kinetics of 8.3-TCR β -transgenic CD8 $^{+}$ T-cells ($n=3$). **B**, Scatchard analysis of tetramer binding to 8.3-TCR β -transgenic CD8 $^{+}$ T-cells ($n=3$). Cells were stained with different concentrations of NRP and NRP-A7 tetramers and the fluorescence units/nM (bound tetramer/free tetramer) plotted against fluorescence units (bound tetramer). At the same concentration, the NRP tetramer occupies fewer T-cell receptors on 8.3-CD8 $^{+}$ T-cells (EU) than the NRP-A7 tetramer. **C**, NRP-A7 tetramer association and dissociation kinetics for islet-derived CD8 $^{+}$ T-cells from pre-diabetic NOD mice ($n=9$, 7 and 13 for K_D , $n=11$, 7 and 12 for $t_{1/2}$ and $n=6$, 7 and 12 for $K_D:t_{1/2}$ ratios at 9, 15 and 20 week-old time points, respectively). **D**, Values for cells from 9 vs. 20 week-old mice: K_D , $p<0.026$ (1 vs. 2); $t_{1/2}$, $p<0.027$ (3 vs. 4); $t_{1/2}:K_D$ ratio, $p<0.015$ (5 vs. 6). The correlation coefficients for the average decay slopes (regression from 0-90 minutes) of samples from 9, 15 and 20 week-old mice were: 0.907 ($p<0.048$); 0.902 ($p=0.054$); 0.993 ($p<0.004$). There were statistical significant differences for the average of the \ln of the normalized fluorescence at 90 minutes between the 9 and 20 week age groups (4.03 ± 0.08 vs. 4.29 ± 0.08 , $p<0.013$). **D**, "avidity maturation" of NRP-A7-reactive cells (mean \pm s.e.m.) vs. diabetes penetrance.

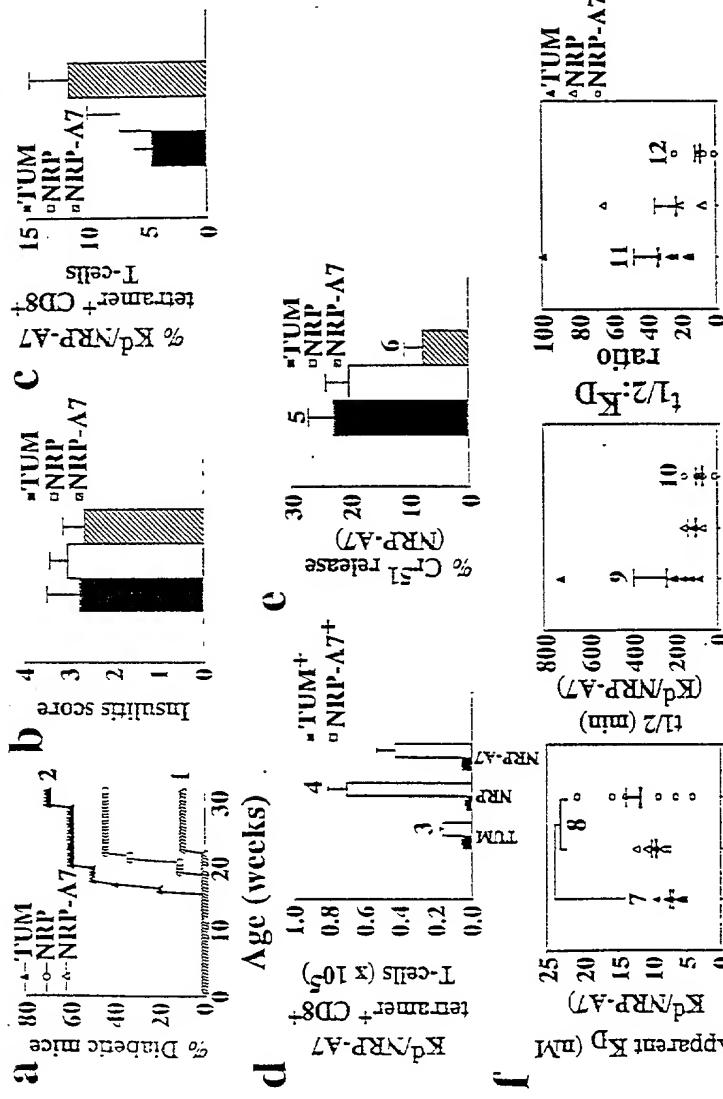


Fig. 12. Diabetogenesis in peptide-treated NOD mice. **A.** Incidence of diabetes in groups of 10 female NOD mice treated with intraperitoneal injections of TUM, NRP and NRP-A7 peptides in PBS. 1 vs. 2, $p<0.007$. **B.** Insulitis scores in non-diabetic mice ($n=2-3$ mice per group; mean \pm s.e.m.). **C.** Percentage and **D.** Absolute number of NRP-A7 tetramer-reactive CD8⁺ cells in peptide-treated mice (ip and fp routes; $n=8$ NRP-A7-, 9 NRP- and 10 control peptide-treated mice). 3 vs. 4, $p<0.015$. The percentage and number of CD8⁺ cells isolated from peptide-treated mice were: 74 ± 38 and $4 \pm 0.2 \times 10^5$ (TUM), 90 ± 18 and $5.7 \pm 1 \times 10^5$ (NRP); and 86 ± 38 and $5.3 \pm 0.9 \times 10^5$ (NRP-A7) ($p=0.03$ for NRP- plus NRP-A7-treated vs. control peptide-treated mice). No differences between diabetic and non-diabetic mice were observed. **E.** NRP-A7-specific murine TUM-specific cytotoxicity of CD8⁺ T-cells from peptide-treated mice (mean \pm s.e.m.). No differences between diabetic and non-diabetic mice were noted. $p<0.017$ for 5 vs. 6. **F.** NRP-A7 tetramer binding kinetics of CD8⁺ T-cells from non-diabetic 32 wk-old peptide-treated mice (ip and fp routes; $n=5$ NRP-A7-, 4 NRP- and 6 control peptide-treated mice) (mean \pm s.e.m.). P values: 7 vs. 8, $p<0.05$; 9 vs. 10, $p<0.04$; 11 vs. 12, $p<0.02$. The correlation coefficients for the average decay slopes (0-30 minutes) of samples from control peptide-, NRP- and NRP-A7-treated mice were: 0.954 ($p<0.023$); 0.899 ($p=0.054$); 0.979 ($p<0.03$). The \ln (normalized fluorescence) values at 90 minutes between the control peptide- and NRP-A7-treated groups were 3.86 ± 0.12 vs. 4.31 ± 0.15 ($p=0.055$).

Figure 13

POSITION									
PRO	K-100	Y-100	N-100	A-100	N-100	W-100	F-100	L-100	
PRO	100	100	100	100	100	100	100	100	
PRO	100	100	100	100	100	100	100	100	
PRO	100	100	100	100	100	100	100	100	
PRO	A-34-26-171	G-2124-150	77-50-294						
PRO	50	50	50	6	6	27			
PRO	N-47-26-134	S-47-14-192	G-17-1-172						
PRO	50	50	50	50	50	50			
PRO	50-101-17-141	F-180-39-138	G-1-9-20-181						
PRO	50	63	50	50	50	50	50	50	
PRO	G-17-1217-9	G-187-36-224	G-184-74-790						
PRO	50	50	50	50	50	50	50	50	
PRO		L-V10-19-103							
PRO		50	50	50	50	50	50	50	
PRO		50-10-211							
PRO		50	50	50	50	50	50	50	
PRO		A-84-17-187							
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PRO		G-185-9-13-143							
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PRO		G-185-102-21-252	G-185-13-39-227						
PRO		50	50	50	50	50	50	50	
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Figure 14

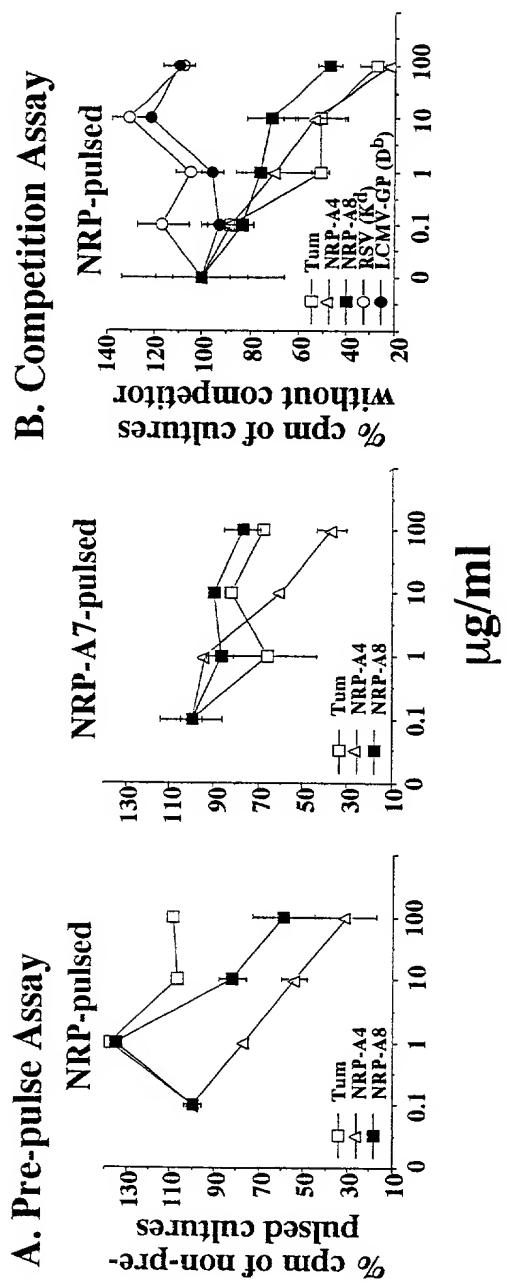


FIGURE 15

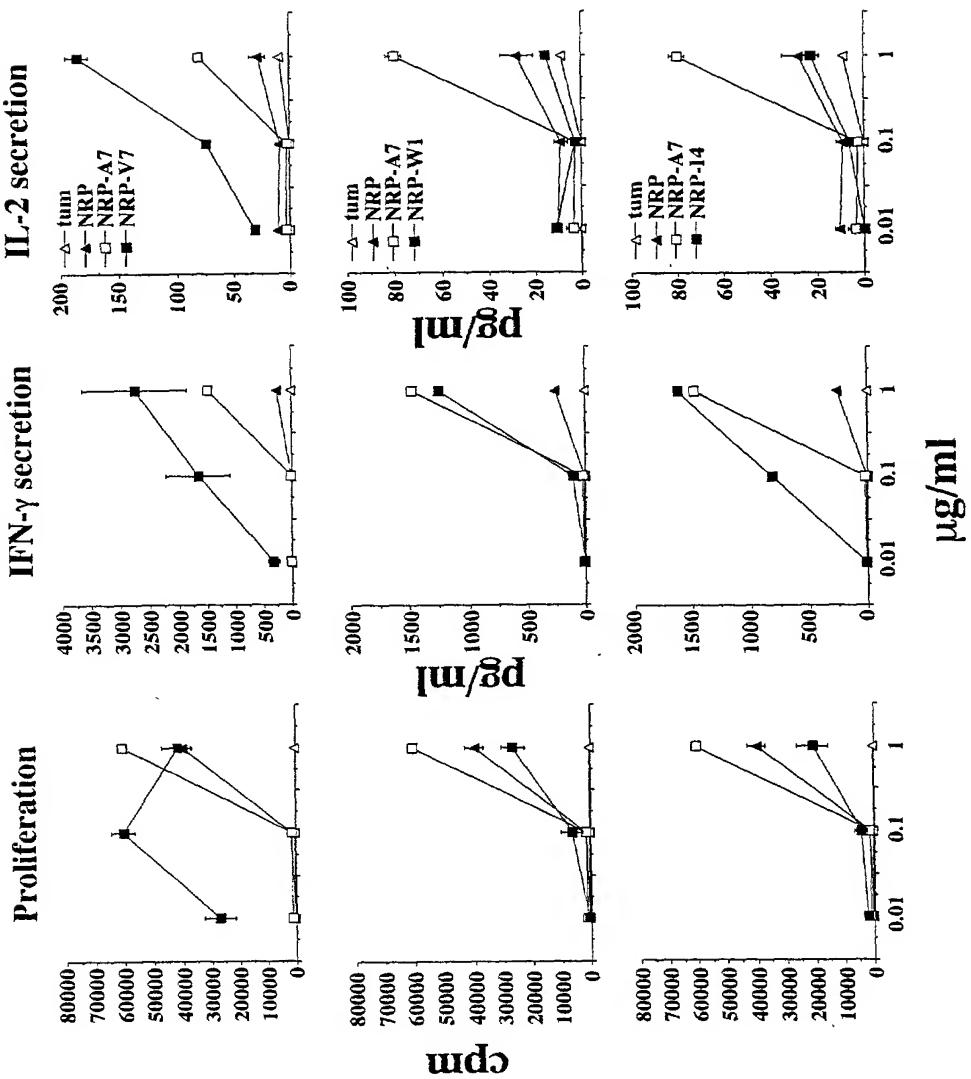


FIGURE 16

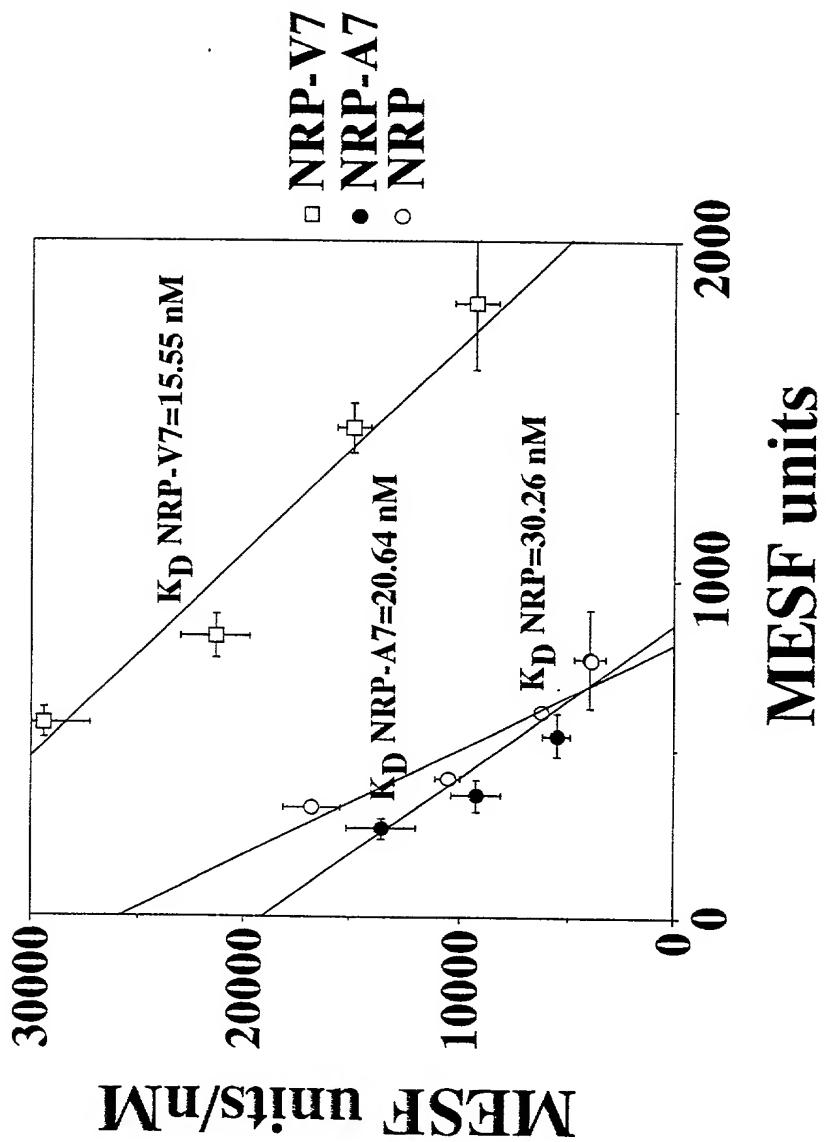


FIGURE 17

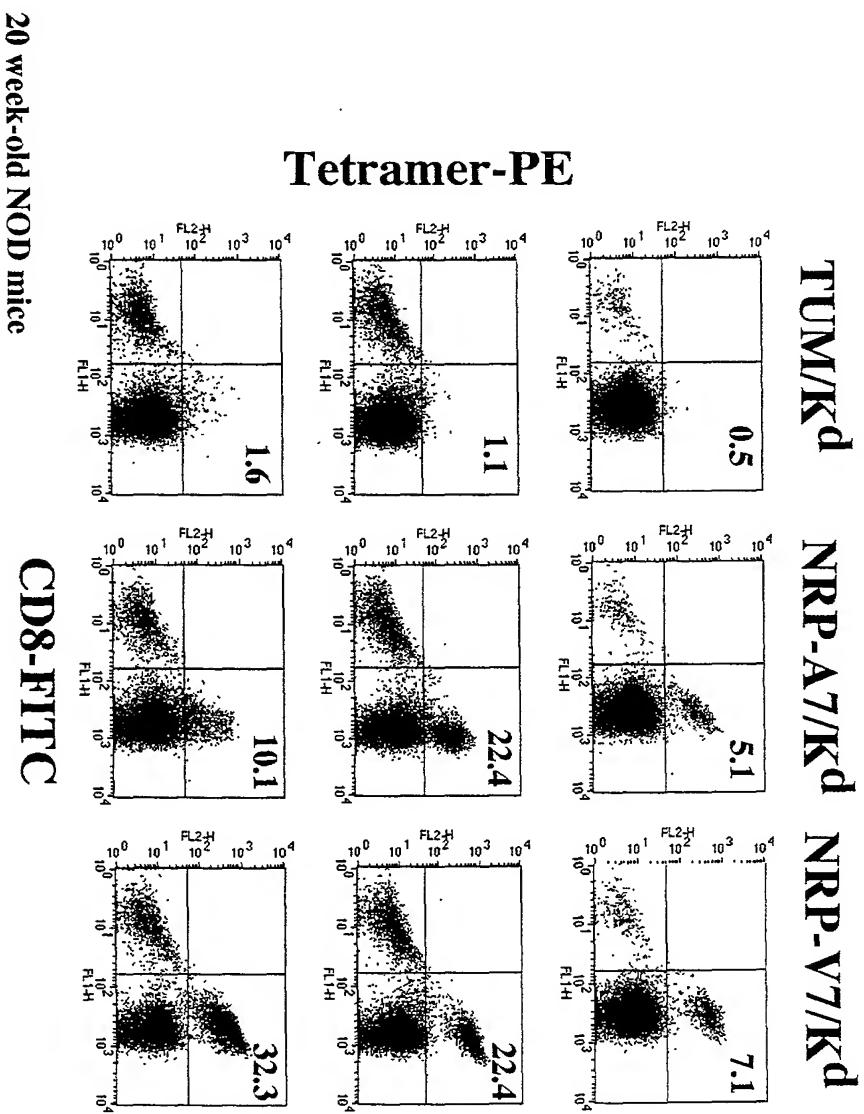


FIGURE 18

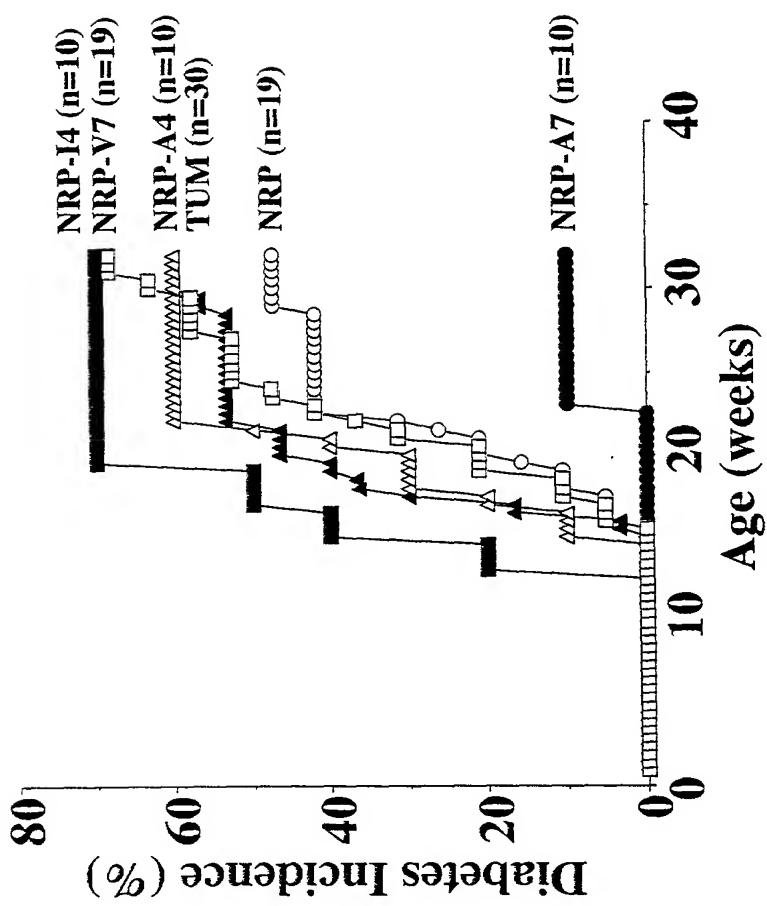
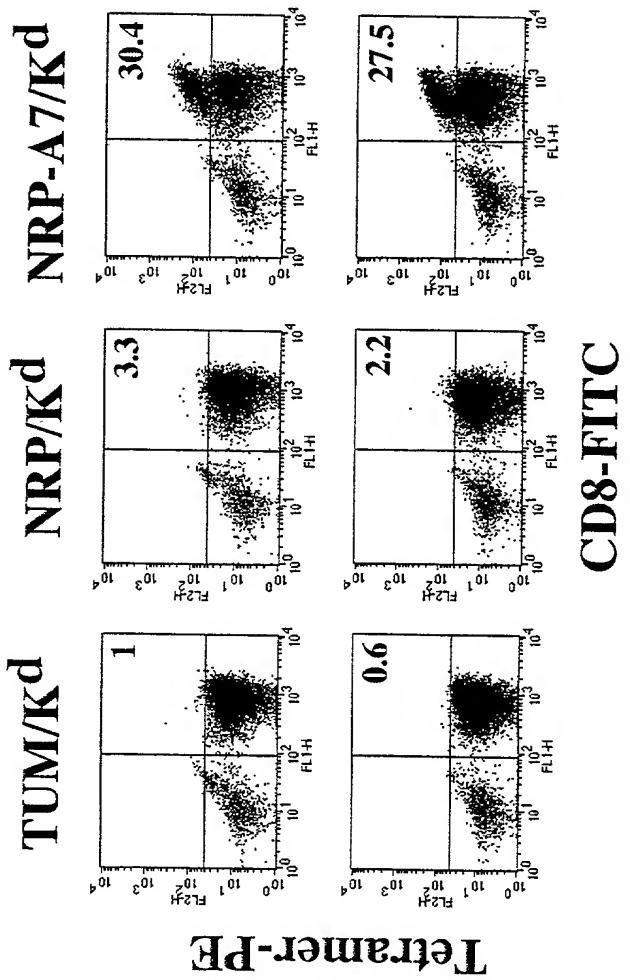


FIGURE 19



NRP-I4-treated NOD mice (@32 wk)

FIGURE 20

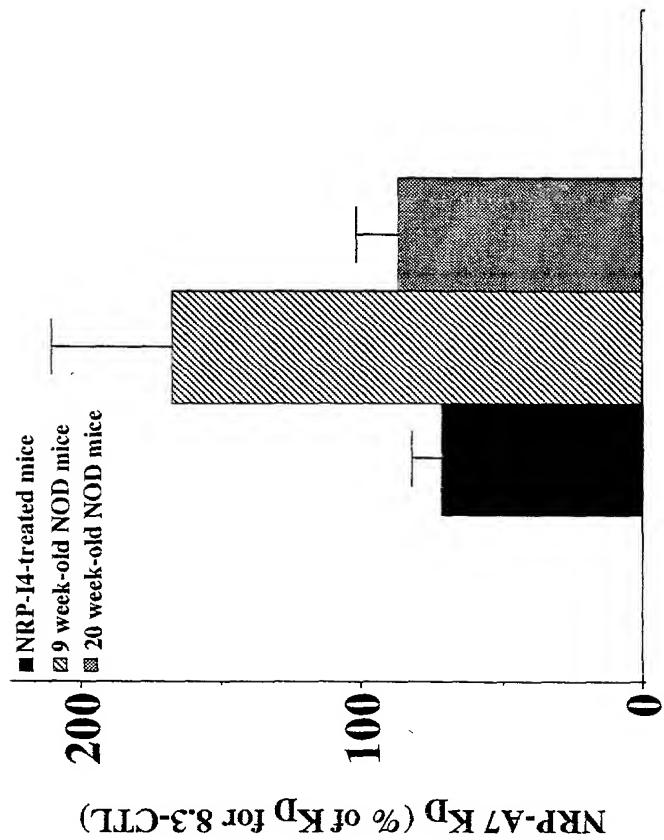
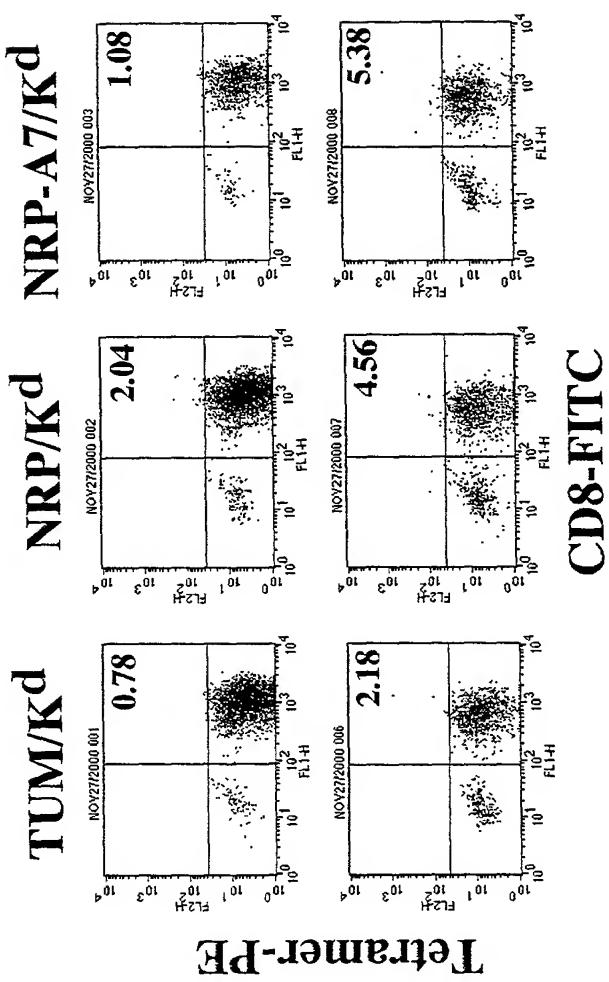


FIGURE 21



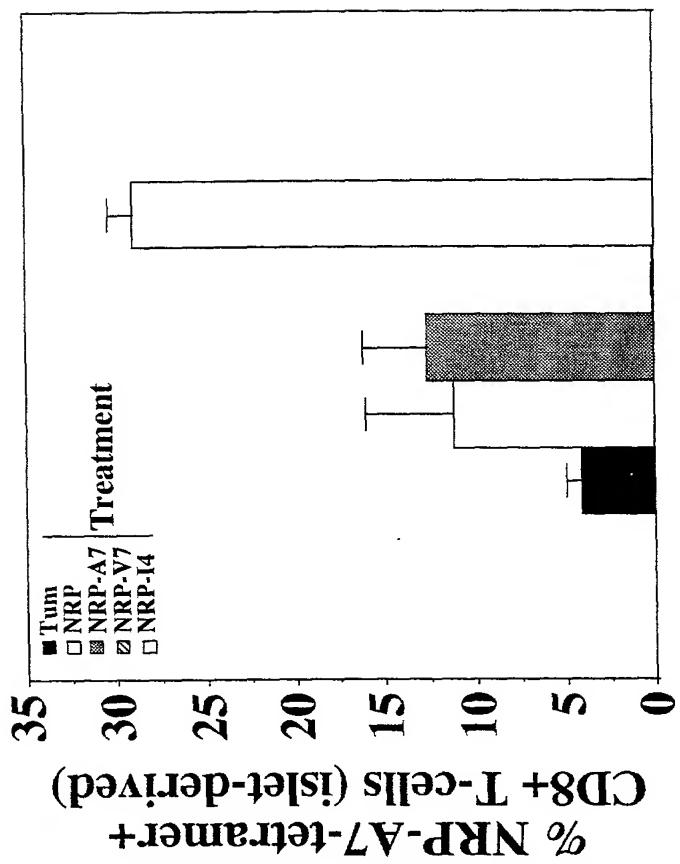
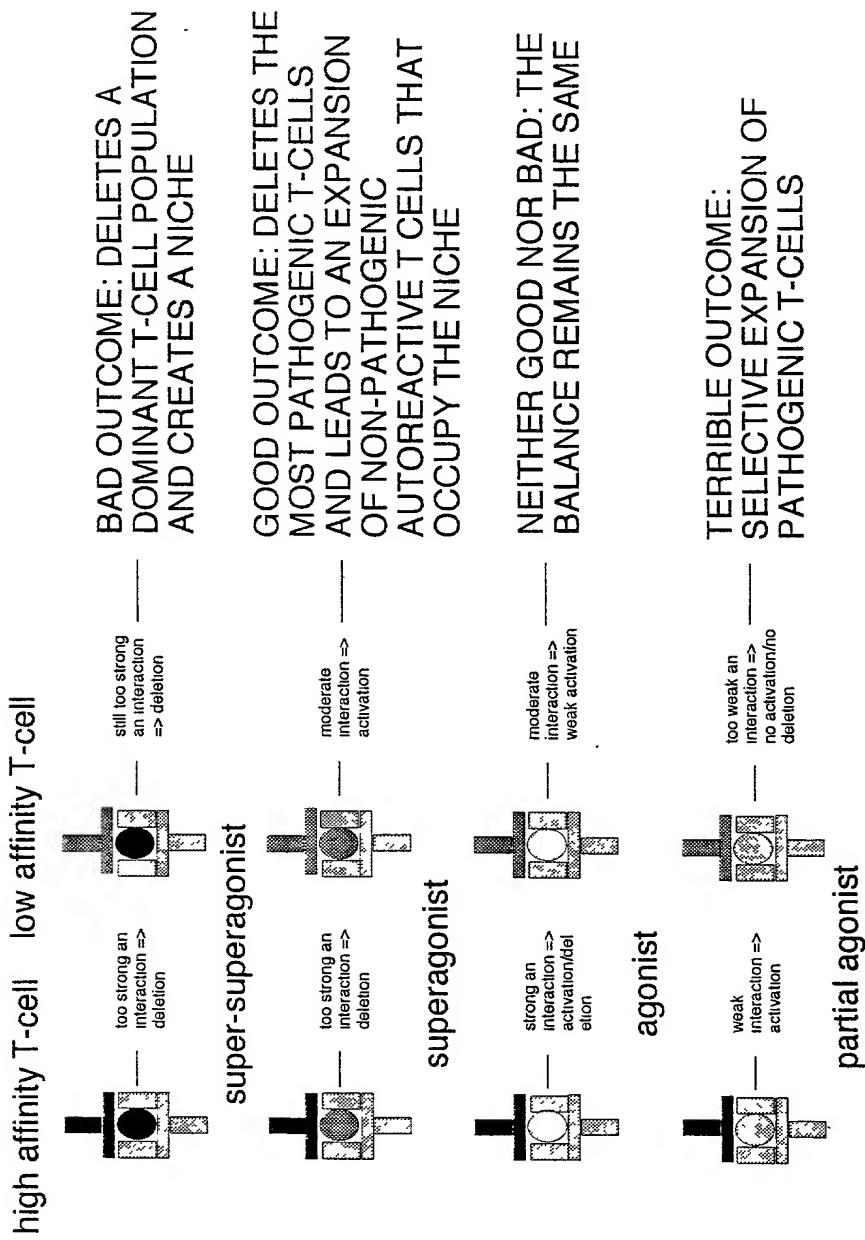
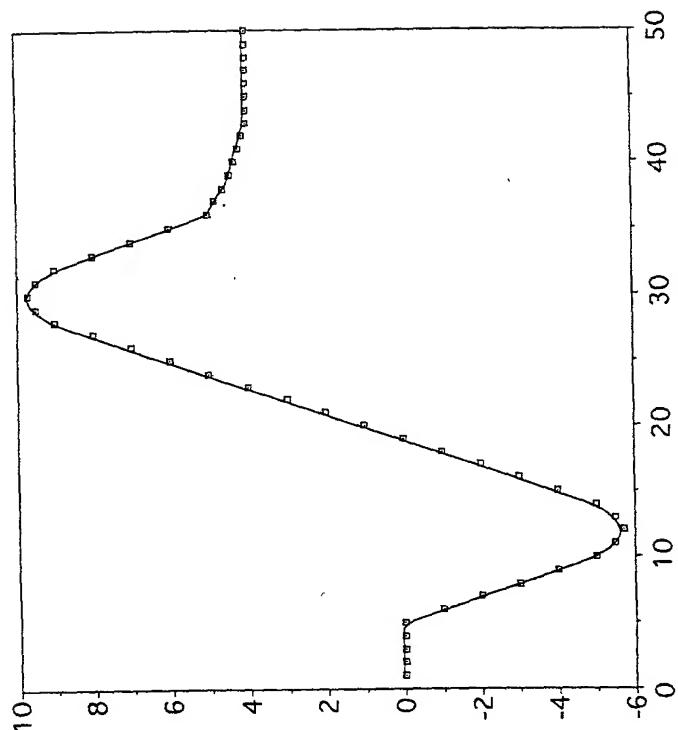


FIGURE 22

FIGURE 23



AFFINITY/AVIDITY

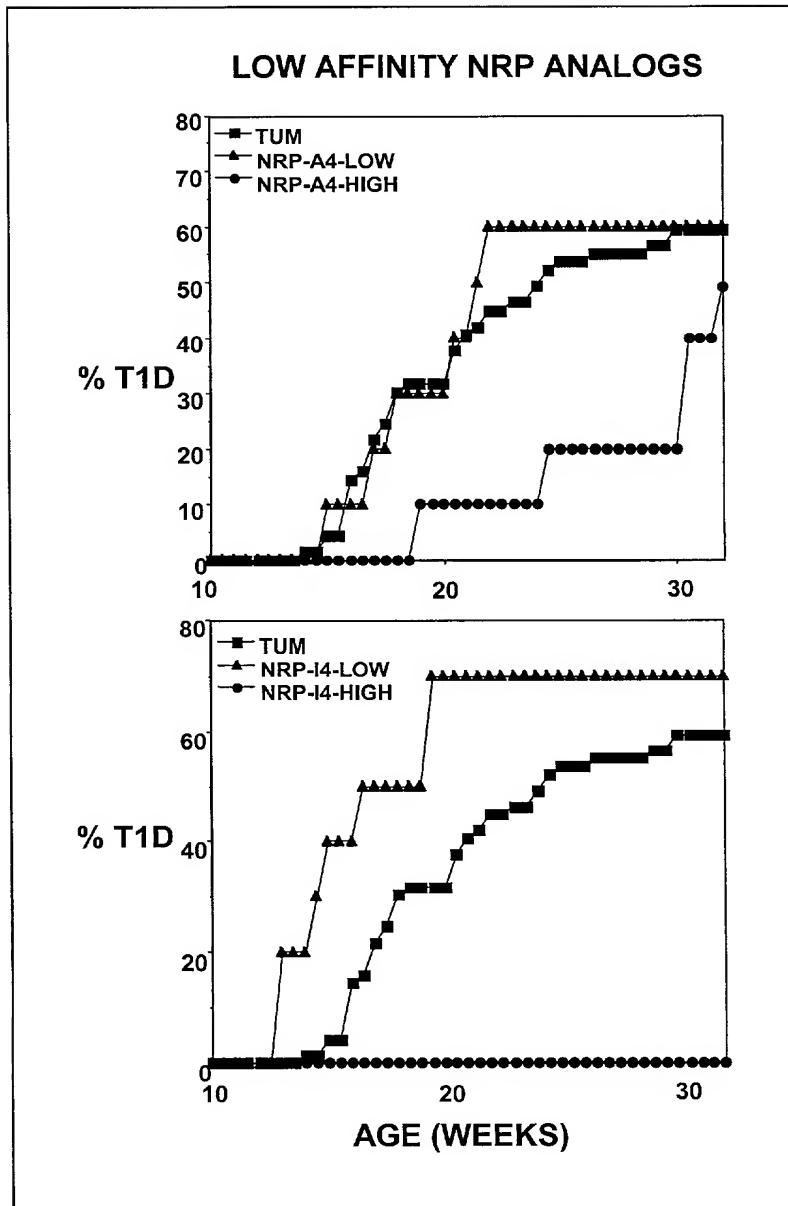


THERAPEUTIC POTENTIAL

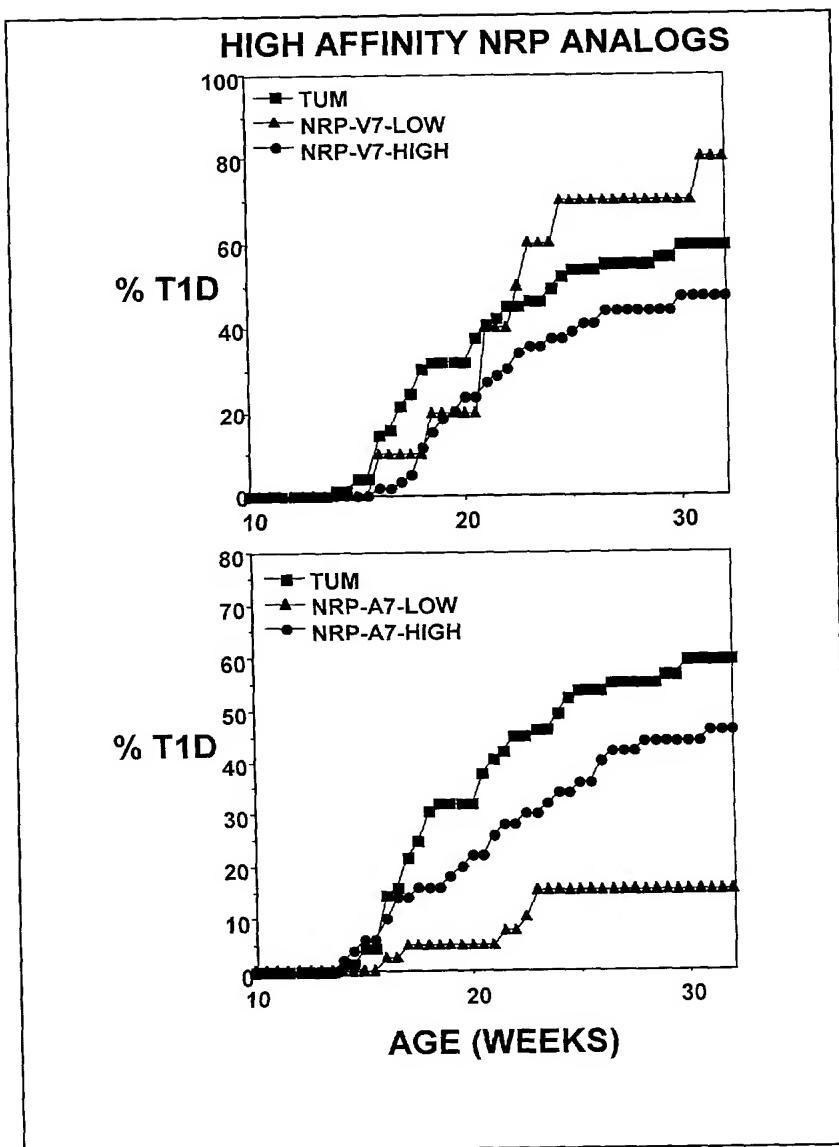
FIGURE 24

APPLN. FILING DATE: HEREWITH
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INVENTOR(S): PERE SANTAMARIE
APPLICATION SERIAL NO: NEW
SHEET 24 OF 28

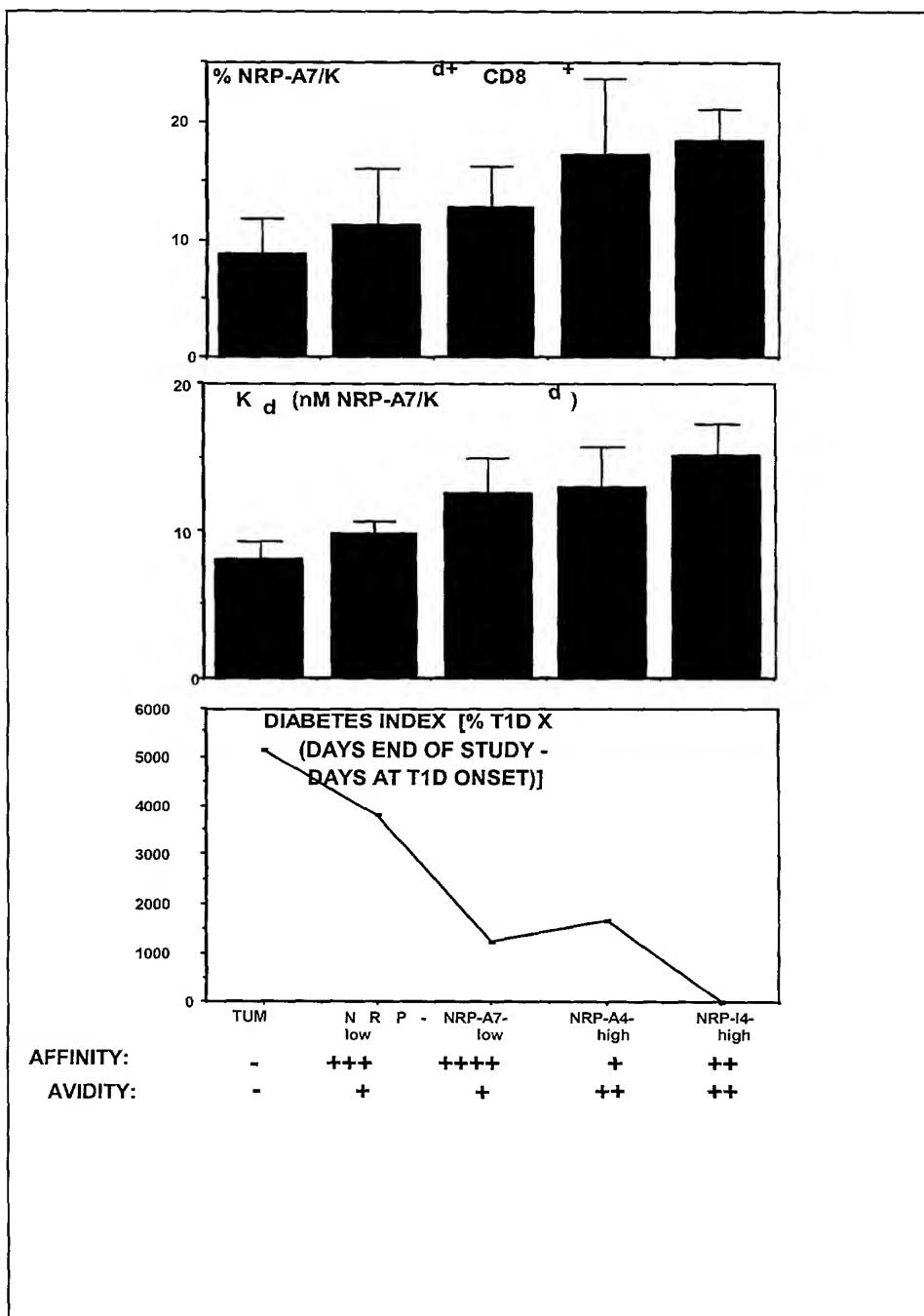
Low affinity NRP mimics and Type 1 Diabetes (T1D)



High affinity NRP mimics and Type 1 Diabetes (T1D)



Anti-diabetogenic regimens and expansion of low avidity cells in islets



Expansion of "irrelevant" autoreactive
cells by elimination of other specificities

